Long-Read Sequencing of Human Cytomegalovirus Transcriptome Reveals RNA Isoforms Carrying Distinct Coding Potentials

Supplementary Material

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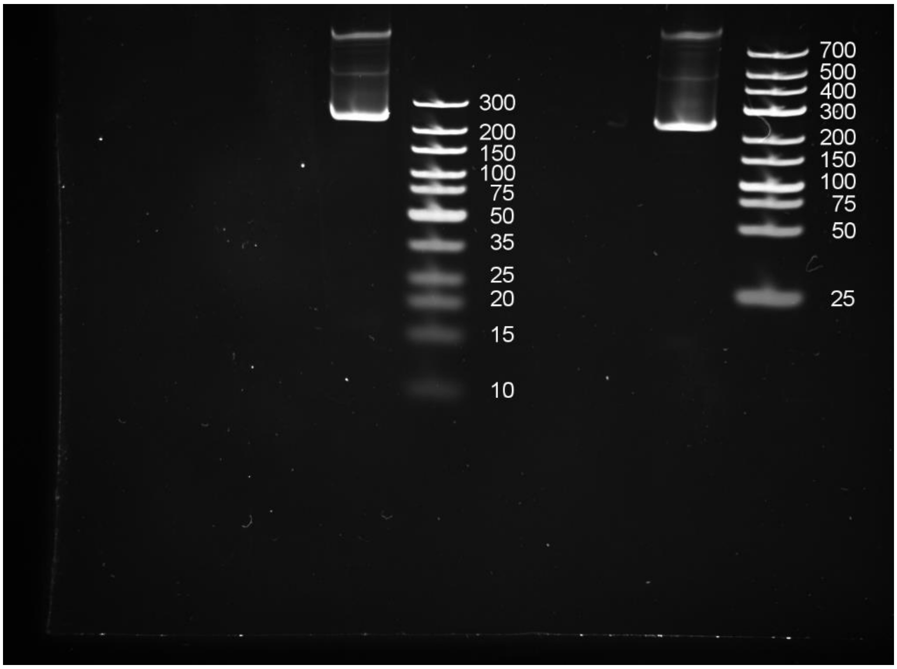
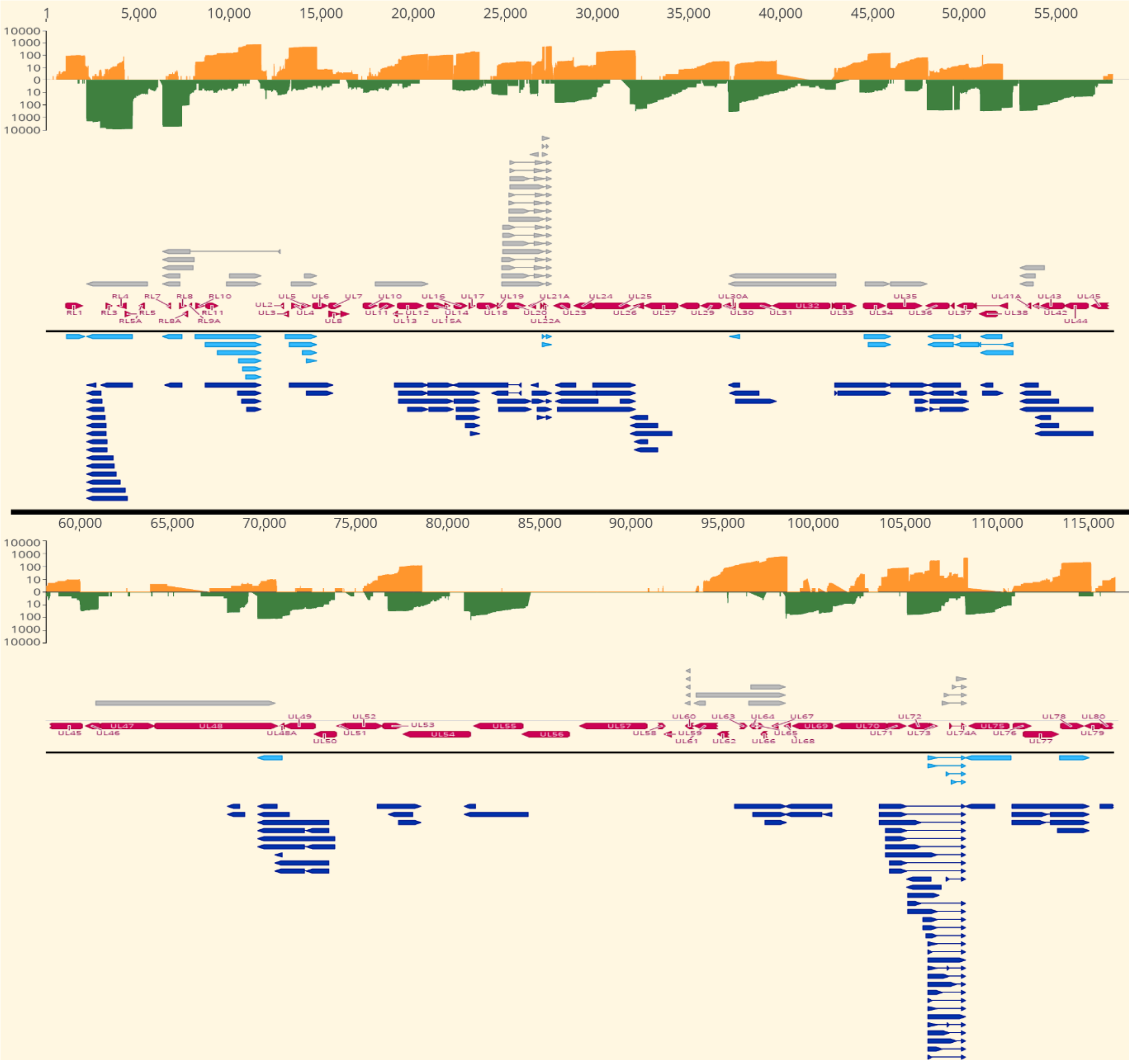
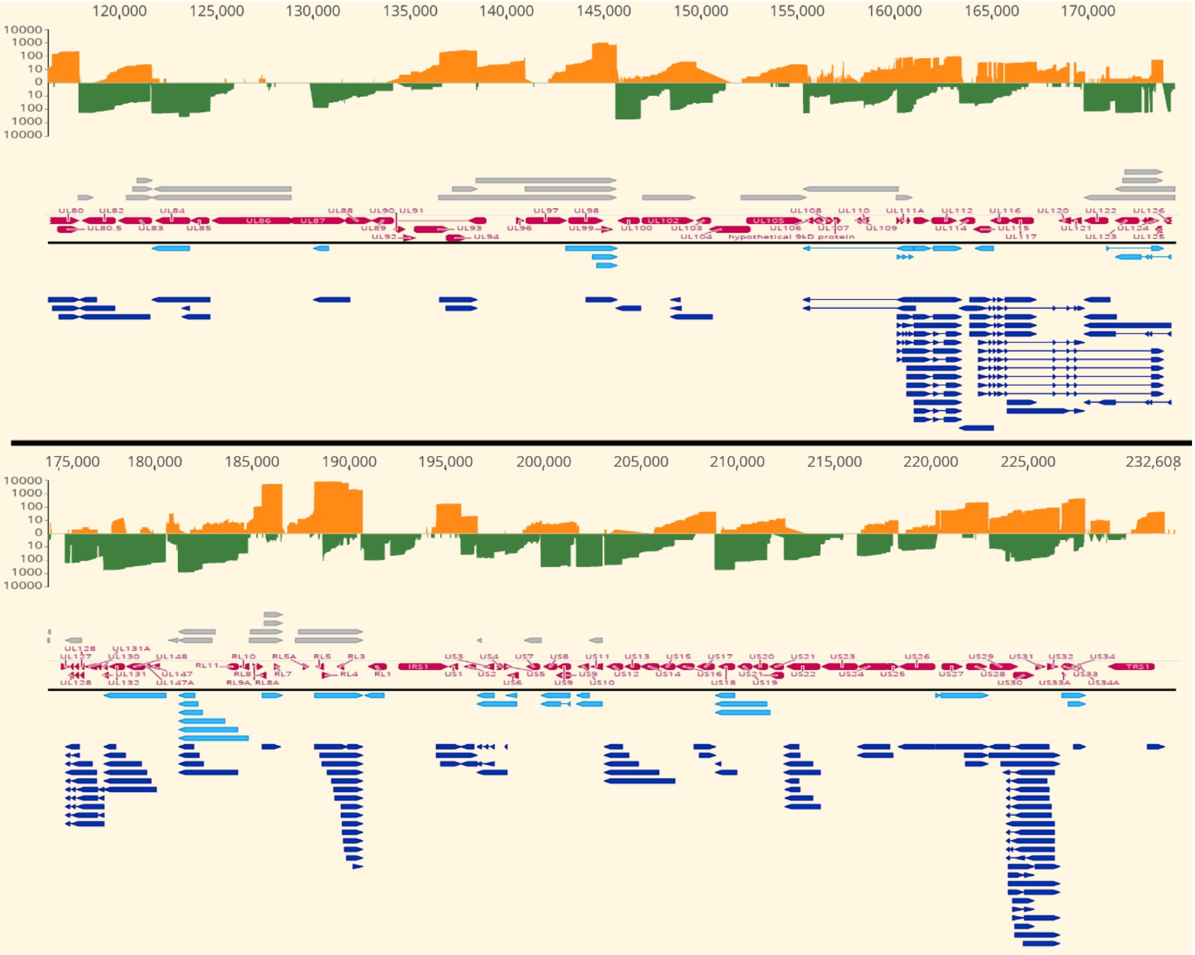
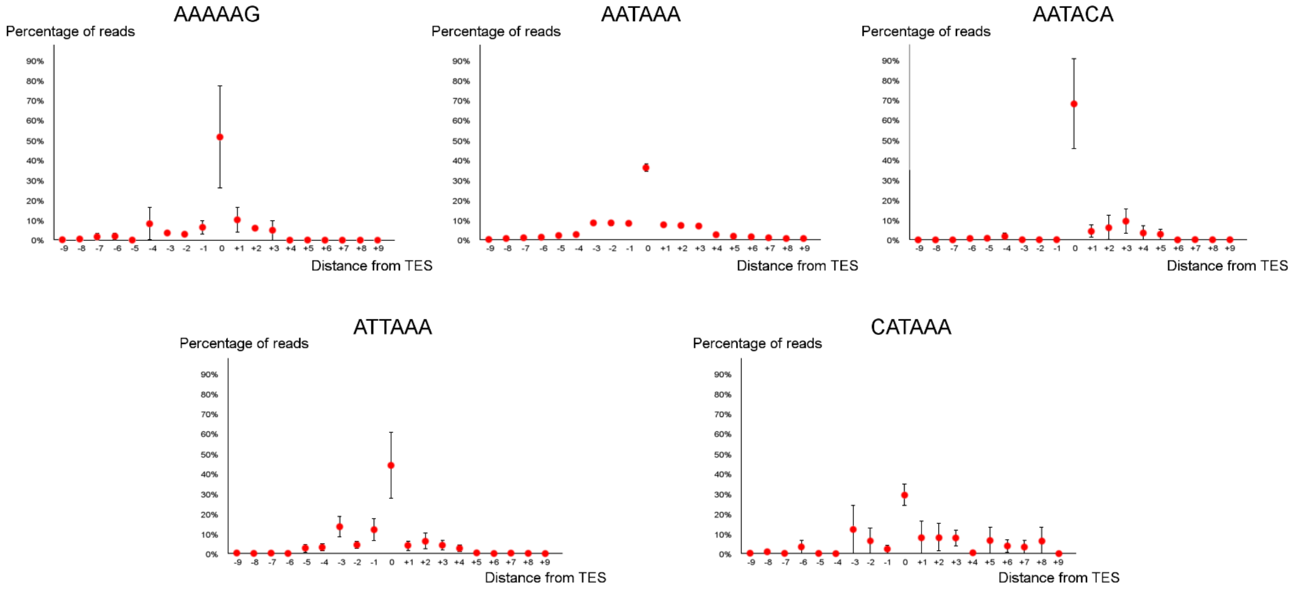


Supplementary Figure S1. The original gel photo used to create Fig. 1 Panel B. Molecular-weight size markers for GeneRuler Ultra Low Range DNA Ladder (left) and for GeneRuler Low Range DNA Ladder (right) are annotated. The image was cropped, and rotated to the left to produce the picture in Fig. 1.

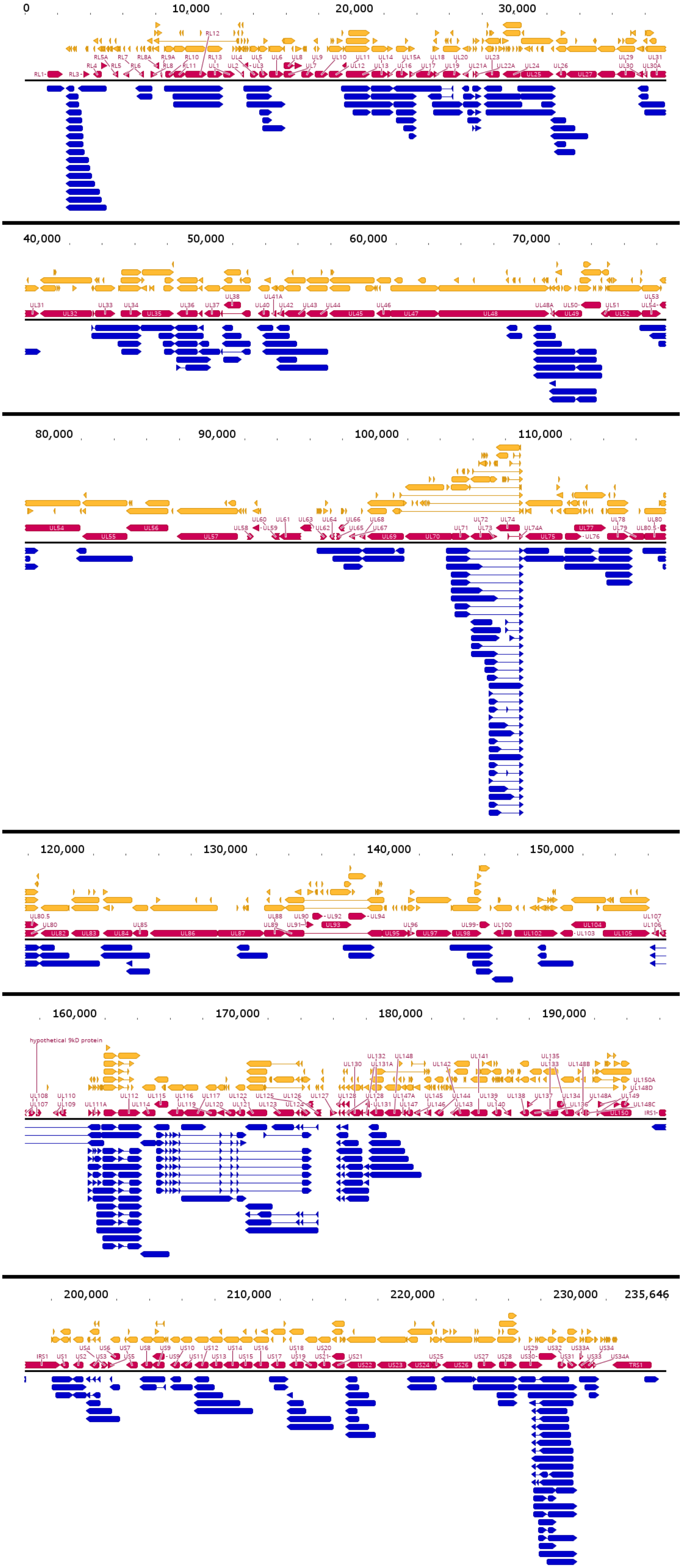




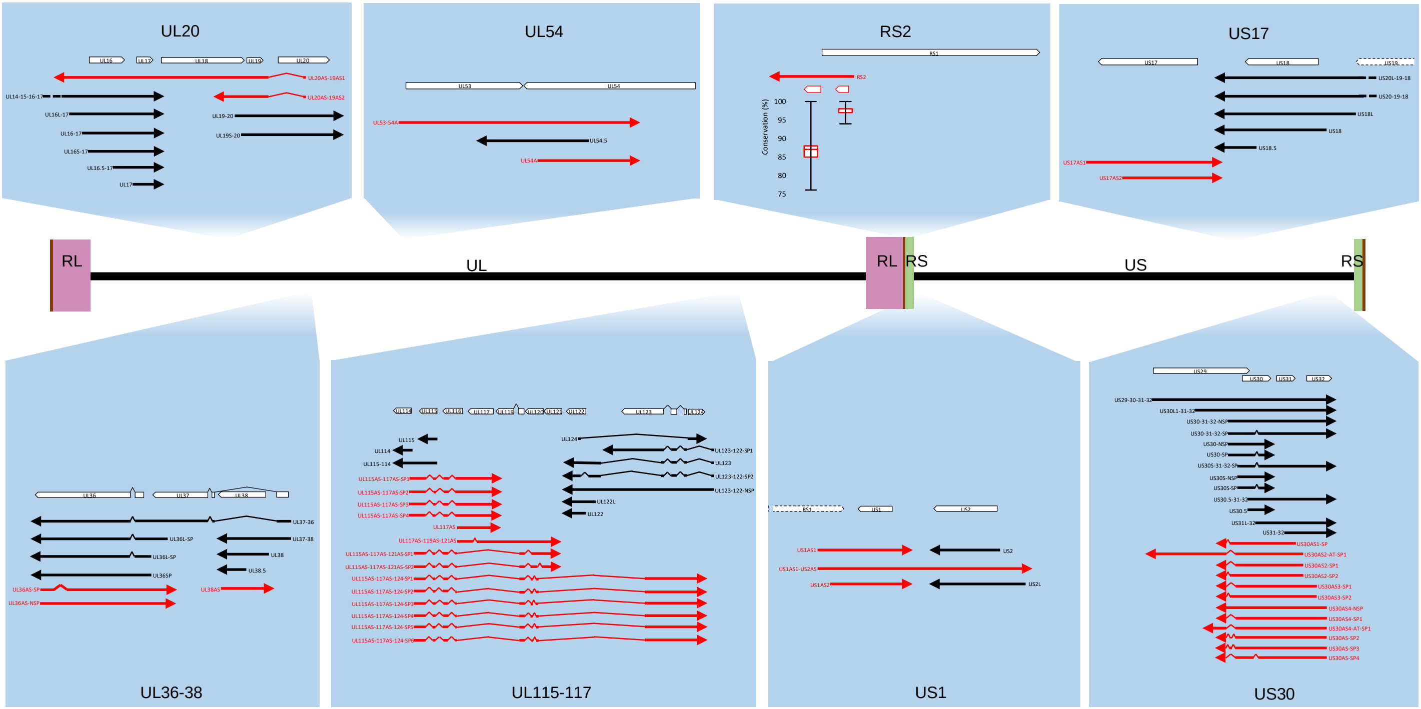
Supplementary Figure S2. Transcriptional landscape of the HCMV. (continued from the previous page) The figure presents the coverage and the annotated transcripts on the genome in four blocks. The coverage histogram (above) is drawn on a logarithmic scale, where the orange bars represent coverage on the plus strand and dark green bars represent coverage on the minus strand. The coverage values of oriented reads from the random and poly(A)$^{+}$ libraries are summed. The annotated transcripts are located below the coverage chart. The previously described transcripts that were not detected by our RNA-sequencing are grey, while the previously described transcripts that our experiments have confirmed have been depicted in light blue, and novel transcript isoforms have been labelled with dark blue. Between the transcripts, canonical ORFs are indicated with purple.



Supplementary Figure S3. Different polyadenylation signals lead to different read ending distribution around TESs. The scatter plot shows the frequency of reads ending in the vicinity of a TES depending on the sequence of the polyadenylation signal. Error bars represent standard errors.


0
10,000
20,000
30,000
RL12
RL5A RL7 RL8A RL9A RL10 RL13 UL4 UL5 UL6 UL8 UL9 UL10 UL11 UL14 UL15A UL18 UL20 UL23 UL29 UL31
RL4 RL5 RL6 RL8 RL11 UL1 UL2 UL3 UL7 UL12 UL13 UL16 UL17 UL19 UL21A UL22A UL24 UL26 UL30 UL30A
RL1 RL3 UL25 UL27
40,000
50,000
60,000
70,000
UL38 UL41A UL53
UL31 UL33 UL34 UL36 UL37 UL40 UL42 UL43 UL44 UL46 UL48A UL50 UL51 UL54
UL32 UL35 UL45 UL47 UL48 UL49 UL52
80,000
90,000
100,000
110,000
UL60 UL61 UL63 UL64 UL66 UL68 UL72 UL74 UL78 UL80
UL58 UL59 UL62 UL65 UL67 UL71 UL73 UL74A UL79 UL80.5
UL54 UL55 UL56 UL57 UL69 UL70 UL75 UL77 UL76
120,000
130,000
140,000
150,000
UL80.5 UL88 UL90 UL92 UL94 UL107
UL80 UL85 UL89 UL91 UL93 UL96 UL99 UL100 UL104 UL106
UL82 UL83 UL84 UL86 UL87 UL95 UL97 UL98 UL102 UL103 UL105
160,000
170,000
180,000
190,000
hypothetical 9kD protein
UL132 UL148 UL141 UL135
UL130 UL131A UL142 UL133 UL148B
UL150A UL148D
UL108 UL110 UL112 UL115 UL116 UL117 UL122 UL125 UL126 UL127 UL128 UL128 UL147A UL145 UL144 UL139 UL138 UL137 UL134 UL148A UL149
UL107 UL109 UL111A UL114 UL119 UL120 UL121 UL123 UL124 UL131 UL147 UL146 UL143 UL140 UL136 UL148C
UL150 IRS1
200,000
210,000
220,000
230,000
235,646
US4 US6 US7 US8 US9 US10 US12 US14 US16 US18 US20 US29 US32 US33A US34
IRS1 US1 US2 US3 US5 US9 US11 US13 US15 US17 US19 US21 US25 US27 US28 US30 US31 US33 US34A
US22 US23 US24 US26 TRS1

Supplementary Figure S4. The annotated transcripts and translationally active ORFs. The transcripts identified by our sequencing experiments (blue) and the ORFs with a significant ribosome footprint as described by Stern-Ginossar et al.[7] (orange) are depicted on the genome map of HCMV strain Merlin (NC_006273). In the middle, canonical ORFs are indicated with purple.



Supplementary Figure S5. Schematic representation of the genomic regions containing the novel transcripts. The HCMV genome is situated in the middle with brown, pink and green rectangles marking the repeat sequences a, b, and c, respectively. The genomic regions containing the novel transcripts are magnified. Canonical ORFs (thick, black arrows) are displayed above the identified transcripts (thin arrows). Novel transcripts are depicted as red. The boxplot shows the nucleotide conservation of the two (not canonical) ORFs found in the transcript RS2 (thick, red arrows). The whiskers represent the range of the data.

Supplementary Dataset S1. Genomic regions that produce artificially 3'-truncated transcripts. Polyadenylated reads terminated at the listed genomic positions, which contain stretches of 3 or more (A)s and therefore they are likely to be false TESs. Coordinates are referenced according to the LT907985 HCMV Towne genome.

Supplementary Dataset S2. Transcriptional end sites. Annotated TESs and the numbers of reads confirming these are listed along with the corresponding polyadenylation signals. Polyadenylation signals were predicted in the genomic sequences 1-50 nt upstream of the TESs. TESs that belonged to an annotated transcript carries their respective names, whereas TESs without corresponding transcripts have been numbered. Coordinates are referenced according to the LT907985 HCMV Towne genome.

Supplementary Dataset S3. Transcriptional start sites. Annotated TSSs and the numbers of reads confirming them are listed along with their distance from the nearest TATA boxes (if applicable). TATA boxes were predicted in the genomic sequences 1-50 nt upstream of the TSSs. TSSs that had belonged to an annotated transcript, carry its name, whereas TSSs without a corresponding transcript numbered. Coordinates are referenced according to the LT907985 HCMV Towne genome.

Supplementary Dataset S4. Deletions breaking the GT/AG rule. The genomic positions of intron-like deletions that did not adhere to the GT/AG rule are listed along with the nucleotide sequences of the donor and acceptor sites. All of these deletions contained 3-6-nt-long repeat sequences (occurring upstream of the donor and downstream of the acceptor sites or downstream of the donor and upstream of the acceptor sites). Coordinates are referenced according to the LT907985 HCMV Towne genome.

Supplementary Dataset S5. Splice junctions. Annotated splice junctions are listed, as well as repeat sequences that may serve as catalysts for template switching. The numbers of reads confirming a given splice site are given for the random and the poly(A) selected libraries as well. Reference numbers for known splice junctions indicate publications where they have been described. Coordinates are referenced according to the LT907985 HCMV Towne genome.

Supplementary Dataset S6. HCMV transcripts and transcript isoforms. Transcript locations are given in GenBank format to include not only TSSs and TESs but also strand and intronic data. The number of reads that contained all features of a transcript isoform (TSS, TES and all splice junctions) are also included along with a relative abundance expressed in percentage compared to the total number of annotated reads. In the case of splice isoforms where this number is 0, the splicing patterns were determined by reads that never reached both the TSS and the TES at the same time. Reference numbers for known transcripts indicate publications where they have been described. Coordinates are referenced according to the LT907985 HCMV Towne genome.

Supplementary Dataset S7. Primers used in the study. The table lists the primer sequences used for the confirmation of the genomic rearrangement and for the RT-qPCR analyses.

References used in the Supplementary Material

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